Salmonella serovar distribution and risk factors associated with persistence of shedding in finishing pigs

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Abstract

The objectives of this study were to describe the *Salmonella* serovar distribution and to identify risk factors associated with serovar persistence in finisher pigs. A longitudinal study was conducted in 18 cohorts of pigs. Fecal culture and serotyping were conducted using standard methods. Among the 446 *Salmonella* isolates (total 187 pigs), there were 18 distinct serovars. The six most common serovars were *S.* Derby (47.3%), *S.* Agona (27.4%), *S.* Johannesburg (10.5%), *S.* Schwarzengrund (2.7%), *S.* Litchfield (2.5%) and *S.* Mbandaka (2.2%). Survival analysis techniques, Kaplan-Meier methods and Log rank test were used to estimate the duration of *Salmonella* shedding in days and test differences in shedding by site, sex, serovar and nursery and environment *Salmonella* status. Cox proportional hazard models, including a shared frailty for cohort, were used to analyze the effect of the risk factors (sex, site, nursery status, environment status, and cold exposure) on duration of *Salmonella* shedding. Overall, the Kaplan-Meier median duration of fecal *Salmonella* shedding was 28 days, and the maximum 112 days. The median duration of shedding in *S.* Derby was 28 days and 14 days for *S.* Agona and *S.* Johannesburg. There was a significant difference of duration of shedding among sites, nursery and environmental *Salmonella* status. Site and nursery status were significantly associated with *Salmonella* shedding. These preliminary results suggest that duration *Salmonella* shedding might depend on farm site or cohort level risk factors.

Introduction

Salmonellosis remains a major foodborne disease threat to public health in the United States (CDC, 2010). *Salmonella* serovars have been recognized to have differential ability to cause illness in different species (*e.g.,* host-adapted serovars such as Choleraesuis in swine, Typhi in humans and Gallinarum in poultry). There is further evidence that some serovars, although capable of zoonotic transmission, are more commonly associated with certain food animal species as compared to others (e.g., Derby in swine). Multiple *Salmonella* serovars can be isolated within an individual pig (Funk et al., 2000; Gebreyes et al., 2004), within groups of swine sampled at one point in time (Funk et al., 2001; Gebreyes et al., 2004; Rajic et al., 2005), within the same group of swine sampled at different ages/production stages (Funk et al., 2001); and across the same farms sampled over several years (Farzan et al., 2008). Risk factors for persistence of serovars in swine have predominantly focused on environmental contamination or transmission from sows to litters (Funk and Gebreyes, 2004). Dorr et al. (2009) described differential distribution of *Salmonella* genotypes by ecological niches, finding persistence of some genotypes more common in the environment, while other genotypes were more commonly isolated from swine. A few genotypes were identified in both swine and environmental samples. These data suggest differential abilities, at least by genotype, for persistence in different swine associated ecological niches (Dorr et al., 2009). The objectives of this study were: (1) describe *Salmonella* serovar distribution in finishing swine; (2) compare persistence of *Salmonella* by serovar; (3) identify risk factors associated with *Salmonella* persistence.

Material and Methods

The samples were collected from a longitudinal study of growing pigs in a multi-site farrow-to-finish production system (3 finishing sites with 6 cohorts each, 18 cohorts total) located in the Midwestern, United States (Pires et al., 2012). Fifty individual pig fecal samples per cohort were collected and cultured every 2 weeks for 16 weeks (8 collections). Fecal samples were processed and cultured using standard methods described previously (Pires et al., 2012). Identification of serotypes was conducted according to Kauffmann-White scheme and was conducted by MSU Diagnostic Center for Population and Animal Health and NVSL.

Risk factors investigated in this study were: sex (male/female), site, exposure to cold, nursery status and environment *Salmonella* status (Pires et al., 2012). The cold exposure was defined as ordinal variable, number times that the pig was exposed to temperatures below the lower critical temperature of the thermal neutral zone (TNZ) in lag times 12h, 24h, 72h, 1 week and 1 month prior the sampling.

Statistical Analysis:
The relative proportion of serovars-specific to the type of sample was described. To determine measures of serovar persistence on farm, time to event data (days of shedding) was calculated. For individual pigs, the duration of shedding for each new infection was defined as the interval between the sampling date of the first *Salmonella*-positive culture and the sampling date of the last positive culture for an individual pig; 7 days were added to the shedding duration to account for shedding during the half the sampling interval after the last positive culture with the same serovar.

The duration of *Salmonella* shedding (days) was analyzed using Stata (version11.0; StataCorp LP College Station, TX) using parametric Kaplan-Meier survival graphs and semi-parametric Cox proportional hazard models, handling ties with Breslow method. Kaplan –Meier graphs were used to visually assess the survival curves (by risk factor groups and the four major serovars). Testing the differences in survivor functions across groups (site, sex, nursery and environment *Salmonella* status) was evaluated using the Log-Rank test. Cox proportional hazard models, including a shared frailty (random effect to account within-cohort correlation among pigs) for cohort, were used to analyze the effect of the risk factors (sex, site, nursery status, environment status, and cold exposure) on duration of *Salmonella* shedding (non-specific to serovar, i.e., *Salmonella* spp, total 175 pigs). Results were considered statistically significant at p-value<0.05. Model diagnostics for the Cox models were based on Cox-Snell and Schoenfeld residuals. The event of interest was *Salmonella* negative (clearance of *Salmonella*), thus survival time was interpreted as the time period (days) a pig shed *Salmonella*. The hazard ratio was calculated for significant variables and interpreted as the higher the hazard ratio, the higher probability for clearance of the infection (the lower the hazard ratio, the higher probability of *Salmonella* shedding).

Results
Among the 446 *Salmonella* isolates (187 pigs total), there were 18 distinct serovars. The six most common serovars were S. *Derby* (47.3%), *S.* Agona (27.4%), *S.* Johannesburg (10.5%), S. *Schwarzengrund* (2.7%), *S.* Litchfield (2.5%) and *S.* Mbandaka (2.2%) (Table 1). Estimation of shedding duration was determined by the 4 major serovars in the 151 pigs. Estimation of shedding duration was determined by site, sex and nursery and environment *Salmonella* status in 175 pigs. Overall, the Kaplan-Meier median duration of fecal *Salmonella* shedding was 28 days, and the maximum 112 days. The median duration of shedding in *S.* Derby was 28 days and 14 days for *S.* Agona, *S.* Johannesburg and *S.* Schwarzengrund. There was a significant difference of duration of shedding among sites, nursery and environment status but not among serovars or sex (p-value<0.05).

The probability for clearance of *Salmonella* from cohorts with a high *Salmonella* nursery status (positive nursery pools greater than the mean) was 0.66 times lower (hazard risk = 0.65 ; 95% C.I. 0.44- 0.94) than pigs from cohorts with positive nursery pools equal to or lower than the mean. There was significant differences among sites; pigs from site C presented higher probability to clear the infection (shorter *Salmonella* shedding duration) compared to site A. However no difference was found between site A and B (hazard ratio = 0.99; 95% C.I. 0.68-1.47). Environment *Salmonella* status, gender and cold exposure (12h, 24h, 48h, 72h, 1 week and 1month) were not significant predictors of shedding duration in final multivariable models.

Discussion
A number of studies have described *Salmonella* prevalence and serovar distribution, mainly related to risk factor studies in finishing pigs (Funk et al., 2001; Gebreyes et al., 2004; Rajic et al., 2005); but a few have examined the duration of shedding and its association with risk factors. The four most common serovars found in this study have been reported in other studies in North America (Farzan et al., 2008; Funk et al., 2001; Gebreyes et al., 2004; Rajic et al., 2005). A significant difference was observed among sites despite belonging to the same production system and having an identical pig source, feed and overall management procedures. Variability in *Salmonella* prevalence among herds and within the same herd over time has been previously reported (Pires et al, 2013; Funk et al., 2001b; Rajic et al., 2005).

There was a significant influence of nursery status (cohorts with positive nursery pools greater than the mean) on duration of fecal shedding. Pigs entering the finisher from these cohorts were exposed to *Salmonella* in the nursery and may have been shedding at arrival to the finishing barn. It seems that previous infection during the nursery increases the duration of

shedding. Future analysis should be conducted to assess the serovar-specific of nursery on *Salmonella* shedding during the finishing phase.

In this preliminary analysis, cold exposure did not affect the duration of shedding, as opposed to the previous findings on *Salmonella* prevalence (Pires et al., 2013). The variable cold exposure was considered time-constant, future analysis should investigate if the effect varies along the finishing phase, since young pigs are more susceptible to cold stress. In addition, other thermal factors should be investigated such as exposure to heat index and to temperatures above the TNZ.

Conclusion

These results suggest that duration *Salmonella* shedding might depend on farm site or cohort level risk factors. These are preliminary findings. Identification of risk factors associated with duration of shedding may allow more targeted interventions to control Salmonella by evaluation of control measures not only for prevalence reduction, but also to decrease the duration of shedding once exposed.

Table 1: *Salmonella* serovar distribution in a longitudinal study in finishing pigs.

Serovar	Individual fecal pigs (%) (n=446)	Nursery (%) (n=62)	Environment (%)(n=33)	TOTAL
S. Derby	211 (47.3)	23 (37.1)	3 (9.1)	237
*S.*Agona	122 (27.4)	28 (45.2)	4 (12.1)	154
*S.*Johannesburg	47 (10.5)	3 (4.8)	9 (27.3)	59
*S.*Schwarzengrund	12 (2.7)	1 (1.6)	0	13
*S.*Litchfield	11 (2.5)	1 (1.6)	0	12
*S.*Mbandaka	10 (2.2)	0	0	10
*S.*Infantis	6 (1.3)	1 (1.6)	0	7
S. Livingstone	5 (1.1)	0	0	5
S. Bovis-morbificans	4 (0.9)	0	0	4
S. Meleagridis	4 (0.9)	1 (1.6)	0	5
S. Montevideo	4 (0.9)	2 (3.2)	17 (51.5)	23
*S.*Worthington	3 (0.7)	0	0	3
*S.*Typhimurium	2 (0.4)	0	0	2
S. Barranquilla	1 (0.2)	1 (1.6)	0	2
S. Lexington	1 (0.2)	0	0	1
S. Orion	1 (0.2)	0	0	1
S. Paratyphi B, L-tartrate+	1 (0.2)	0	0	1
S. Rough O:b:l,w	1 (0.2)	0	0	1
S. Muenchen	0	1 (1.6)	0	1

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